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Spread of two invasive flies (Diptera: Drosophilidae) infesting commercial fruits in southeastern Brazil

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Drosophila suzukii Matsumura (Diptera: Drosophilidae) is among the most recent invasive insect pests in southern Neotropical countries such as Argentina, Brazil, Chile, and Uruguay (Andrezza et al. 2017). This species is highly polyphagous, and attacks thin-skinned fruits such as blackberry (*Rubus* spp.), blueberry (*Vaccinium* spp.), raspberry (*Rubus idaeus* L.), and strawberry (*Fragaria × ananassa* Duchesne) (Bellamy et al. 2013), as well as many non-crop hosts (Kenis et al. 2016). The larvae hatch from eggs laid under the fruit skin and feed on the fruit, causing it to become unmarketable (Asplen et al. 2015). The first record of *D. suzukii* in the Neotropics was in 2013 in southern Brazil, and was associated with a 30% loss in strawberry production (Deprá et al. 2014; Santos 2014). Despite the widely reported damage capacity of *D. suzukii*, the infested area was not placed under quarantine, and insecticides were not registered for its management in Brazil. This likely contributed to its spread from southern Brazil to Rio de Janeiro (Bitner-Mathé et al. 2014) and Minas Gerais States (Andrezza et al. 2016) in southeastern Brazil (Fig. 1). *Zaprionus indianus* Gupta (Diptera: Drosophilidae) is another invasive fly pest of fruits in South America (Vilela 1999; Fartyal et al. 2014; Bernardi et al. 2017). It has recently been detected in *D. suzukii* monitoring traps in a large number of crops in Canada, Mexico, and the United States (Renkema et al. 2013; Joshi et al. 2014; Lasa & Tadeo 2015). Laboratory studies indicated that *Z. indianus* attacks undamaged strawberries, with increased oviposition associated with previous *D. suzukii* oviposition (Bernardi et al. 2017).

Drosophila suzukii is currently expanding its range in the Neotropics and threatens economically valuable fruit crops in invaded areas. Espírito Santo State, Brazil, borders areas invaded recently by *D. suzukii* in southeastern Brazil (Fig. 1). It has a diverse range of climates, which makes possible the cultivation of both subtropical and tropical fruits, including some of the principal *D. suzukii* hosts and potential *Z. indianus* hosts, such as blackberry and strawberry. Climate-based prediction models indicate there is potential for *D. suzukii* to invade this Brazilian state (Benito et al. 2016). Therefore, an increase in knowledge of the distribution and hosts of both species in southeastern Brazil is needed. To determine the spread of *D. suzukii* in this region, fruit samples were collected in Espírito Santo from blackberry (n = 76), and strawberry (n = 577) fields in the highlands (> 900 masl), papaya (*Carica papaya* L.) (n = 2) and cattley guava (*Psidium cattleianum* Afzel. ex Sabine) (n = 2) at

sea level, and robusta coffee (*Coffea canephora* Pierre ex A. Froehner) (n = 1,085) at intermediate altitudes (100–300 m asl). Fruits were randomly collected from a blackberry field (n = 1) (Field 1: 20.3716667°S, 41.0641667°W, 970 m asl), and several strawberry fields (n = 8) ([Field 2: 20.2541667°S, 40.9905556°W, 1,026 m asl]; [Field 3: 20.3052778°S, 41.0294444°W, 912 m asl]; [Field 4: 20.3763889°S, 41.0305556°W, 1,080 m asl]; [Field 5: 20.1386111°S, 41.0025000°W, 1,050 m asl]; [Field 6: 20.1608333°S, 40.9191667°W, 1,036 m asl]; [Field 7: 20.2194444°S, 40.9850000°W, 1,110 m asl]; [Field 8: 20.0819444°S, 40.8902778°W, 851 m asl]; [Field 9: 20.1744444°S, 40.8563889°W, 962 m asl]) in the municipalities of Domingos Martins and Santa Maria de Jetibá from Jul 2016 to Feb 2017 (Fig. 1). To monitor adult emergence, the fruits were placed on a layer of paper towels inside plastic boxes (20 × 30 × 17 cm) with vents covered with fine mesh, at the Laboratory of Entomology, Federal University of Viçosa (UFV) at 25 ± 2 °C, 50 ± 5% RH and 12 h (L:D) photoperiod. Adult flies were collected daily over a 14 d period, and preserved in 96% alcohol for species identification. Additional samples were collected directly from strawberry canopies using an aspirator. Two collections of flies associated with papaya and *P. cattleianum* fruits that were rotting on the ground in a small garden in the municipality of Serra, Espírito Santo, ([Field 10: 20.2038889°S, 40.1972222°W, 3 m asl]; [Field 11: 20.2038889°S, 40.1975000°W, 3 m asl]) were made in Aug 2017 to determine the presence of *D. suzukii* in a coastal area of the state (Fig. 1). Collections were made directly by quickly placing a large, open, transparent plastic bag over fruit infested with flies and quickly closing the bag as the flies flew upward into the bag, which was then lifted away from the fruit. After being anesthetized inside a freezer, the flies were killed and preserved in 96% alcohol for later identification. Mature berries of robusta coffee were collected in the municipalities of Governador Lindenberg (Field 15: 19.2083333°S, 40.5008333°W, 376 m asl); Linhares (Field 16: 19.3669444°S, 40.4472222°W, 142 m asl); Marilândia (Field 12: 19.4047222°S, 40.5405556°W, 97 m asl); (Field 13: 19.4041667°S, 40.5391667°W, 127 masl); (Field 14: 19.4044444°S, 40.5408333°W, 93 m asl); and Sooretama (Field 17: 19.1219444°S, 40.0819444°W, 61 m asl) (Fig. 1), and maintained in plastic containers as described previously for blackberry and strawberry fruits to collect adult flies that developed in the fruit. *Drosophila suzukii* and *Z. indianus* specimens were identified according to Vilela & Mori (2014), and

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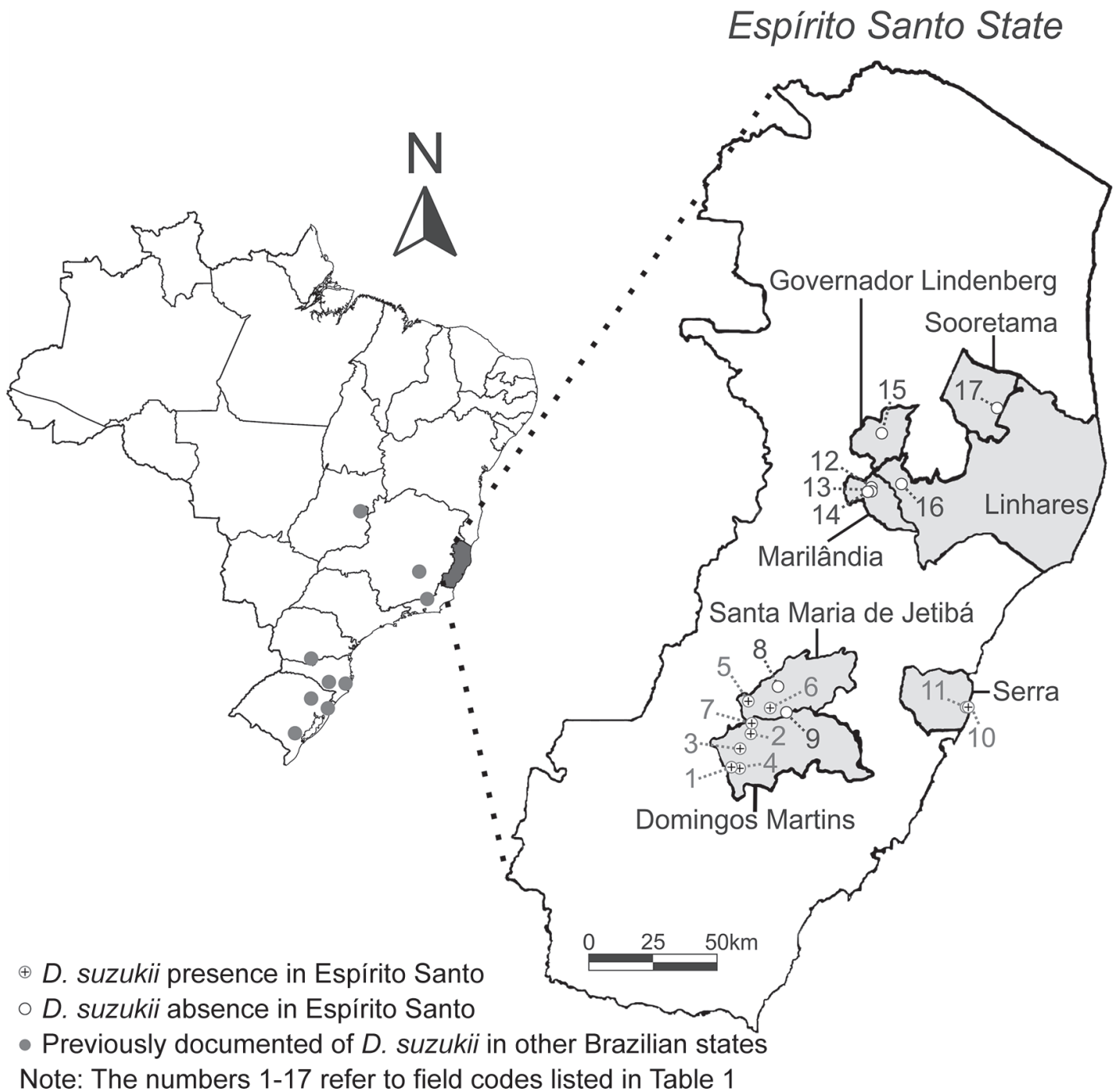


Fig. 1. Brazilian states previously invaded by *Drosophila suzukii* and its new dispersion area in Espírito Santo State, Brazil.

Van der Linde (2010), respectively. Specimens of *D. suzukii* collected in papaya and *P. cattleianum* were sent to the California Department of Food and Agriculture for molecular identification.

In the first yr of collecting (i.e., 2016), *Drosophila* was the most common genus collected (n = 485) in strawberry fields, followed by *Z. indianus* (n = 16). However, no *D. suzukii* specimens were collected during 2016. *Drosophila suzukii* was collected directly only in Feb 2017, as adults (28♂, 8♀) from blackberry fruits in the municipality of Domingos Martins, and this is the first report of *D. suzukii* in Espírito Santo. A total of 965 *D. suzukii* adults (54% females) emerged from the blackberry fruits (n = 76) demonstrating a high infestation in this region

(Table 1). From these blackberry fruits, an additional 458 *Z. indianus*, and 21 *Drosophila* spp. adults also emerged (Table 1). *Drosophila suzukii* emerged from fruits of 6 of the 8 strawberry fields surveyed in 2017 (Table 1), but it was collected directly as adults only from 1 field. *Zaprionus indianus* and *Drosophila* spp. were present in all strawberry fields sampled (Table 1). The former species was first recorded in Espírito Santo in Sep 2004 associated with ripe papaya used as bait to attract flies (Culik 2004). Other species collected in these soft fruits were not identified. In this study, 5 fly specimens associated with papaya and *P. cattleianum* fruit rotting on the ground were collected and identified as *D. suzukii* (Table 1) based on species-specific taxonomic characters

Table 1. Field infestation rates of Drosophilidae occurring in diverse host plants in Espírito Santo State, Brazil.

Fly species/Host Plant	Municipality	Field code	Sample date	Fruits evaluated (N)	Specimens identified (N)	Infestation rate (flies/fruit)	
<i>Drosophila suzukii</i> Matsumura							
Blackberry	Domingos Martins	1	Feb/2017	76	445 ♂, 520 ♀	12.70	
Strawberry	Domingos Martins	2	Feb/2017	74	5 ♂, 10 ♀	0.20	
		3	Feb/2017	110	4 ♂, 1 ♀	0.05	
		4	Feb/2017	63	1 ♂, 2 ♀	0.05	
		5	Feb/2017	61	0 ♂, 2 ♀	0.03	
	Santa Maria de Jetiba	6	Feb/2017	79	2 ♂, 1 ♀	0.04	
		7	Feb/2017	66	1 ♂, 4 ♀	0.08	
		10	Aug/2017	1	3 ♂	3.00	
Papaya ¹	Serra	10	Aug/2017	1	3 ♂	3.00	
Papaya and <i>P. cattleianum</i> ¹	Serra	11	Aug/2017	NC ²	2 ♂	—	
<i>Zaprionus indianus</i> Gupta ³							
Blackberry	Domingos Martins	1	Feb/2017	76	458	6.03	
Strawberry	Domingos Martins	2	Feb/2017	74	369	4.99	
		3	Feb/2017	110	204	1.85	
		4	Feb/2017	63	12	0.19	
		5	Feb/2017	61	341	5.59	
	Santa Maria de Jetiba	6	Feb/2017	79	793	10.04	
		7	Feb/2017	66	758	11.48	
		8	Feb/2017	63	172	2.73	
		9	Feb/2017	61	346	5.67	
<i>Drosophila melanogaster</i> Meigen							
<i>Coffea canephora</i>	Marilândia	12	May/2012	210	1 ♂, 4 ♀	0.02	
		13	May/2012	181	2 ♂, 11 ♀	0.07	
		14	May/2012	129	2 ♂, 4 ♀	0.05	
		15	May/2012	217	4 ♂, 2 ♀	0.03	
	Governador Lindenberg	Linhares	16	May/2012	65	1 ♂	0.01
		Sooretama	17	May/2012	300	4 ♂, 5 ♀	0.03
<i>Drosophila ananassae</i> Doleschall							
<i>Coffea canephora</i>	Governador Lindenberg	15	May/2012	83	3 ♂, 4 ♀	0.08	

Note: Each row in the table corresponds to a sampled field. ¹fruits rotting on the ground; ²NC = fruits not counted; ³this species was not sexed.

including the distinct black spot on the wings, and tarsal combs on the legs (Hauser 2011). Also, the 2 specimens sent to the California Department of Food and Agriculture were confirmed as *D. suzukii* based on morphological characteristics and CO1 molecular analysis [collection data: Manguinhos, Serra, Espírito Santo, Brazil; 5 Aug 2017; col. MP Culik; ex. *Carica papaya* fruit, mature or rotting on ground (1 male); Manguinhos, Serra, Espírito Santo, Brazil; 12 Aug 2017; col. MP Culik; ex. *C. papaya* and *P. cattleianum* fruit, mature or rotting on ground (1 male)]. Six samples of robusta coffee berries were infested by drosophilid flies, but none with *D. suzukii* or *Z. indianus*; *Drosophila ananassae* Doleschall (3 ♂, 4 ♀) was associated with this coffee species in Governador Lindenberg, and *Drosophila melanogaster* Meigen (14 ♂, 26 ♀) was found in all coffee fields sampled (Table 1). However, these species associated with robusta coffee have been of low agricultural importance, and frequently are associated with post-harvest fruits and advanced ripening. They are cosmopolitan species of wide geographic distribution found more frequently in domestic environments and rarely in natural habitats (Bock & Parsons 1978; Singh & Yadav 2015).

Results of this study confirm the continued spread of the *D. suzukii* in the Neotropics. Its current distribution is verified now in the highlands and coastal areas of Espírito Santo with high levels of infestation found in blackberry fruits, a known host of *D. suzukii* (Bellamy et al. 2013; Burrack et al. 2013). Although strawberry has been reported as a host of *D. suzukii* in the Neotropics (Bernardi et al. 2017), the low infestation level found in this study may indicate that *D. suzukii* is in an initial stage of invasion in this crop. *Zaprionus indianus* has previously

been shown to oviposit in undamaged strawberry fruits under laboratory conditions (Bernardi et al. 2017), but has not previously been reported infesting undamaged blackberries. *Zaprionus indianus* not only was present in high numbers in the present study, but also has been recently noted under field conditions, and in traps from several other regions, including Asia and North America (Fartyal et al. 2014; Joshi et al. 2014; Lasa & Tadeo 2015). Thus, the infestation abilities of *Z. indianus* in undamaged or *D. suzukii*-damaged blackberry fruits should be further investigated in laboratory oviposition studies, and in more extensive field surveys. Other *D. suzukii* fruit hosts, including grapes, loquats, and raspberries were not evaluated in the present study, but they also are cultivated economically in Espírito Santo, and they are hosts of *D. suzukii*. Tropical fruits such as papaya also are important economically in this Brazilian state, so laboratory susceptibility tests with tropical fruit species and other potential new hosts also should be done. Such research efforts will facilitate the ability to predict and delay further dispersal of *D. suzukii* and similar invasive pests throughout this and other Neotropical areas.

Findings from this survey also highlight the continued lack of effective mitigation actions to stop *D. suzukii* spread within Brazil. Even after being demonstrated by Vilela & Mori (2014) that the fresh market of blueberries allowed *D. suzukii* flies to be transported across several states in Brazil, its spread and damage capacity apparently remain neglected. The pest presence in the region, coupled with the development of appropriate management methods, should be widely reported to soft fruit growers, who can increase the monitoring ef-

forts through the active daily scouting of the fields, for example, and take early management actions for its control. This improvement in the communication among producers, researchers and extension agents should, at least partially, reduce the spread and the economic losses caused by these invasive species.

Summary

Two invasive fruit flies, *Drosophila suzukii* Matsumura and *Zaprionus indianus* Gupta (Diptera: Drosophilidae), recently have become established, and are expanding their range in the Neotropics. This research documents the range expansion of these species in southeastern Brazil, where they were found infesting cultivated blackberries and strawberries in the highlands of Espírito Santo, Brazil. *Drosophila suzukii* also was found associated with rotting *Psidium cattleianum*, and for the first time associated with papaya at sea level. *Drosophila suzukii* and *Z. indianus* may further affect establishment and production of important soft fruit crops and papaya in this region of Brazil. Management methods for fruit production in this Brazilian region need to be improved to avoid damage and further spread of *D. suzukii*.

Key Words: blackberry; *Drosophila suzukii*; invasive pests; papaya; strawberry; *Zaprionus indianus*

Sumário

Duas espécies invasivas de moscas-das-frutas, *Drosophila suzukii* Matsumura e *Zaprionus indianus* Gupta (Diptera: Drosophilidae) recentemente se estabeleceram e estão expandindo sua distribuição na região Neotropical. Esta pesquisa documenta a expansão de áreas afetadas por estas duas espécies no sudeste do Brasil, onde foram encontradas infestando amora preta e morango economicamente cultivados na região serrana do Espírito Santo, Brasil. *Drosophila suzukii* também foi encontrada associada a frutos de *Psidium cattleianum* em decomposição e pela primeira vez, associada a frutos de mamão, ao nível do mar. *Drosophila suzukii* e *Z. indianus* podem afetar o estabelecimento e a produção de importantes culturas de pequenas frutas e mamão nesta região do Brasil. Métodos de manejo para produção de frutas nesta região brasileira precisam ser melhorados para evitar danos e a dispersão de *D. suzukii*.

Palavras Chaves: amora preta; *Drosophila suzukii*; mamão; morango; pragas invasivas; *Zaprionus indianus*.

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